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論文題目	Role of Lignin in Nutritional Physiology of a Lower Termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) （イエシロアリの栄養生理におけるリグニンの役割）		

（論文内容の要旨）

The mechanisms of the decomposition of polysaccharides by termites have been well-documented. However, there is almost no information regarding the role of lignin in the nutritional physiology of wood-feeding insects. Studies on the role of lignin in the nutritional physiology of wood-feeding insects would improve our understanding of natural lignocellulose-decomposition mechanisms, and contribute to the development of sustainable technology to convert lignocellulosic biomass to valuable fuels and materials.

The present study therefore aimed to investigate the role of lignin in nutritional physiology of a lower termite, *Coptotermes formosanus*.

In Chapter 1, the lignocellulose deconstructions by a lower termite, *C. formosanus*, with emphasis on the decomposition of lignin polymers were discussed. Japanese cedar (*Cryptomeria japonica*) (softwood), Japanese beech (*Fagus crenata*) (hardwood), and rice straw (*Oryza sativa* L. ssp. *japonica* cv. Nipponbare) (grass) samples were fed to *C. formosanus* workers, and the fecal materials were subjected to detailed structural analyses using high-resolution 2D NMR as well as a series of wet-chemical analyses. High-resolution NMR structural data suggested preferential removal of syringyl aromatic units in hardwood lignins, but non-acylated guaiacyl units as well as tricin end-units in grass lignins. In addition, the data suggest that termites and/or their gut symbionts may favor degradation of C–C-bonded β–5 and resinol-type β–β lignin inter-monomeric units over degradation of ether-bonded β–*O*–4 units, which is in contrast to what has been observed in typical lignin biodegradation undertaken by wood-decaying fungi.

Chapter 2 investigated the chemical composition in each lignocellulose, and the physiological responses of a lower termite *C. formosanus* fed various lignocelluloses and purified lignins (milled-wood lignins, MWLs) from Japanese cedar, Japanese beech, and rice straw to determine the physiological effects of different lignocellulose sources and lignins on *C. formosanus* such as termite survival, body mass, and the changes of the symbiotic protists in the hindgut of workers. The results suggested that the survival, body mass and presence of both *Pseudotrichonympha grassii* and *Holomastigotoides hartmanni* in the hindgut of workers fed on rice lignocellulose at the 4th week of observation were significantly lower than those of the workers fed on Japanese cedar and Japanese beech

lignocellulose samples, whereas there was no significant difference in *Spirotrichonympha leidyi* among all the diets. The three purified MWLs, regardless of their structural differences, did not show any significant differences for the termites' survival or body mass or the survival of all the three protists. The three MWL diets resulted in significantly lower termite survival compared to starvation, although these diets showed no significant effects on body mass or the protist profiles. Overall, lignins are hardly utilized as a nutrient source by *C. formosanus* workers and are even rather detrimental to termites when fed on solely.

Chapter 3 further investigated the effects of lignin on the nutritional physiology of *C. formosanus*. In this chapter, hydrogen and methane emissions from *C. formosanus* workers fed various lignocelluloses, and MWLs, and artificial diets composed of mixtures of MWLs and polysaccharides (holocellulose or cellulose) were measured. The results showed that H_2 and CH_4 emission rates of workers fed artificial diets composed only of isolated lignin polymers, regardless of the different plant sources, were in general similar to those by starved control workers. In addition, no significant difference in H_2 and CH_4 emission rates was observed between diet groups containing polysaccharides with lignin and those containing only polysaccharides. These data suggest that lignins have little effect on the activity of the symbiotic micro-organisms that support lignocellulose decomposition in the hindgut of *C. formosanus* workers, at least under the present test conditions (4 weeks incubation).

In Chapter 4, the effects of lignins as diet components on the physiological activities of a lower termite, *C. formosanus* were investigated to determine the exact role of lignin in termites. The artificial diets composed of polysaccharides (holocellulose or α-cellulose) with and without MWLs) from Japanese cedar, Japanese beech, and rice straw were fed to *C. formosanus* workers. The survival and body mass of the workers and the presence of three symbiotic protists in the hindguts of the workers were observed. The survival rates of workers fed on diets containing lignins were, regardless of the lignocellulose diet sources, significantly higher than those of workers fed on only polysaccharides. In addition, it was clearly observed that all the tested lignins have positive effects on the maintenance of two major protists in the hindguts of *C. formosanus* workers, i.e., *P. grassii* and *H. hartmanni*. Overall, the data suggest that the presence of lignin is crucial to maintaining the physiological activities of *C. formosanus* workers during their lignocellulose decomposition.

Chapter 5 reported the effects of dietary lignin on bacterial community profiles in the hindgut of a lower termite *C. formosanus*. In the experiments for this chapter, diet samples were prepared only from Japanese cedar, and the bacterial community was profiled using ARISA (Automated Ribosomal Intergenic Spacer Analysis). The results suggested that

lignin when served as a sole food had apparently little effect on the bacterial pools in the hindgut of *C. formosanus* workers. However, fractional analysis on ARISA profiling data obtained for workers fed polysaccharide diets with and without lignins suggested that lignin when served with polysaccharides has a marked effect on the bacterial pools in the hindgut of *C. formosanus* workers. Taken together with the previous findings in Chapter 4, the results obtained in this chapter support the view that lignin plays an important role in maintaining gut microbial communities during lignocellulose digestion in lower termites' digestive systems.

Overall, the results support the view that lignin polymers are partially decomposed during their passage through the termite gut digestive system. It was also found that lignins give marked positive effects on the survival of *C. formosanus* workers as well as on their maintenance of hindgut protists when served with polysaccharide diets. Furthermore, it was suggested that the gut bacterial community profiles are also affected by dietary lignins. This study has provided evidence that the presence of lignin is crucial to maintaining the physiological activities and a wholesome hindgut digestive system of *C. formosanus* workers for their efficient lignocellulose decomposition.

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（続紙 ２ ）

（論文審査の結果の要旨）

シロアリは自然界におけるリグノセルロースの循環において非常に重要な位置を占める生物であるとともに、木材・木質材料の最重要害虫としてその防除が図られている。シロアリによるセルロースの分解機構については、これまでの多くの研究によって、シロアリ、原生動物、および細菌類の３者による巧妙な消化共生系が実証されてきているが、自然界でセルロースに次いで大量に存在するリグニンについては、その分解機構および栄養学的な意味は全く明らかになっていない。本論文は、世界的な重要害虫であるイエシロアリを対象とし、高分子リグニンの分解と栄養生理における役割を先端的化学分析と生物試験によって詳細に検討したものであり、特に評価すべき点として以下の３点を挙げることができる。

１．スギ、ブナおよびイネという３つの異なったリグニン構造をもつリグノセルロースをイエシロアリに摂食させ、摂食前後のリグニン構造の変化を２次元NMRによって詳細に分析することにより、ブナのシリンギルユニット、並びにイネのグアイアシルおよびトリシンユニットが減少することを明らかにし、さらに、白色腐朽菌類による場合とは異なり、β-βおよびβ-5構造がβ-*O*-4構造に優先して分解を受ける事を示した。これは、全く新しいリグニン分解系の存在を示唆する重要な結果である。

２．精製した高分子リグニン（MWL）を単独でイエシロアリに与えた場合、栄養学的にはむしろ有害であることを示すとともに、ホロセルロースあるいはセルロースとともに与えると、長期的にはシロアリの生存や消化管内共生原生動物相の健全な維持に有益であることを詳細な観察により実証した。すなわち、シロアリに対するリグニンの栄養学的な意味を初めて明らかにしたものである。

３．ARISA法を消化管内共生細菌相の解析に応用することによって、イエシロアリにホロセルロースあるいはセルロースを単独で、またはMWLと混合して与えた場合、長期的には両者間で消化管内細菌相に明確な違いが生じることを明らかにし、リグニンのシロアリ消化管内細菌相への影響を初めて示した。

以上のように、本論文はシロアリによる高分子リグニンの分解を初めて実証するとともに、リグニンの存在がシロアリの栄養生理、特に消化管内に共生する微生物相に対して重要な意味を有することを明らかにしたものであり、昆虫生理学、木質バイオマス化学、および木材保存学の発展に寄与するところが大きい。

よって、本論文は博士（農学）の学位論文として価値あるものと認める。

なお、平成３０年２月８日、論文並びにそれに関連した分野にわたり試問した結果、博士（農学）の学位を授与される学力が十分あるものと認めた。

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